Seasonal variation of potential denitrification rates of surface sediment from Meiliang Bay, Taihu Lake, China

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Abstract

The regulatory effects of environmental factors on denitrification were studied in the sediments of Meiliang Bay, Taihu Lake, in a monthly sampling campaign over a one-year period. Denitrification rates were measured in slurries of field samples and enrichment experiments using the acetylene inhibition technique. Sediment denitrification rates in inner bay and outer bay ranged from 2.8 to 51.5 nmol N_2/(g dw (dry weight)·hr) and from 1.5 to 81.1 nmol N_2/(g dw·hr), respectively. Sediment denitrification rates were greatest in the spring and lowest in the summer and early autumn, due primarily to seasonal differences in nitrate concentration and water temperature. For each site, positive and linear relationships were regularly observed between denitrification rate and water column nitrate concentration. Of various environmental factors on denitrification that we assessed, nitrate was determined to be the key factor limiting denitrification rates in the sediments of Meiliang Bay. In addition, at the two sites denitrification rates were also regulated by temperature. The addition of organic substrates had no significant effect on denitrification rate, indicating that sediment denitrification was not limited by organic carbon availability in the sediments. Nitrate in the water column was depleted during summer and early autumn, and this suppressed effective removal of nitrogen from Taihu Lake by denitrification.

Key words: denitrification; sediment; environmental factors; Taihu Lake

DOI: 10.1016/S1001-0742(09)60205-9

Introduction

In the last decades, N-loading of inland waters has steadily increased globally due to a number of factors, such as increased fertilizer application, change in land use, discharge of untreated domestic wastewater, and atmospheric deposition (Torre et al., 1992; Pattinson et al., 1998). Nutrient enrichment can cause eutrophication in freshwater leading to algal blooms, reduced oxygen concentration, and an alteration in ecosystem structure and function (Carpenter et al., 1998). Understanding the mechanisms that control N-cycling in aquatic ecosystems is of fundamental importance to aquatic ecology, particularly in regions where nutrient loading is great. Thus, it is increasingly important to understand the processes that lead to a removal of nitrate from aquatic systems.

Denitrification is the microbially mediated process whereby NO_3^- is reduced to N_2O or N_2, and heterotrophic bacteria, i.e., denitrifiers, are the dominant organisms responsible for this process (Payne, 1973). Denitrification can play an important role in the nitrogen budgets of lakes (Cavari and Phelips, 1977). Sediment denitrification is of particular importance in the context of N export, because it represents a permanent removal of N from the aquatic ecosystem (Seitzinger, 1988; Sjodin et al., 1997).

Taihu Lake is the third largest freshwater lake in China with a surface area of 2338 km^2 and an average depth of about 2 m (Chen et al., 2003), where, increased nutrient inputs related to population and economic growth have led to eutrophication. Most pollutants come from rivers discharging into Meiliang Bay and other parts of the Taihu Lake (Huang, 2000). Meiliang Bay is one of the most eutrophied bays in the northern part of Taihu Lake. Blooms of blue-green algae have been frequently occurring in warmer seasons (Pu et al., 1998). Eutrophication studies in Taihu Lake often focus on phosphorus, since it is the most common limiting nutrient. Most studies in this lake have been limited to monitoring nutrient sources and nutrient concentration; whereas studies on nitrogen cycling processes in Taihu Lake are scarce (Yang et al., 1998; McCarty et al., 2007). There is not yet enough information available about denitrification in the sediments of Taihu Lake.

The primary goal of this study was to quantify potential denitrification rates in the sediments of Meiliang Bay, Taihu Lake, and to assess how various environmental factors affecting denitrification. We choose Meiliang Bay

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as research object, partly because it is one of the most eutrophied bays in the northern part of Taihu Lake, with high nitrogen loading in the water column.

1 Materials and methods

1.1 Site description

We selected two sites within Meiliang Bay located in the inner Meiliang Bay (inner bay; "A" in Fig. 1) and outer Meiliang Bay (outer bay; "B" in Fig. 1). Site A (31°31′33.9″N, 120°12′35.2″E) is located in a key state tourism area, and is subject to intensive recreational usage. Untreated sewage effluent is discharged into Meiliang Bay via outfall of Liangxihe River, located about 2 km from site A. Liangxihe River is a main river running through Wuxi City. Site B (31°25′49.3″N, 120°12′27.7″E) is located in the inlet of Meiliang Bay, and receives a greater proportion of nitrogen loading from the mainly agricultural catchment, rather than from sewage or industry.

1.2 Field sampling

We conducted monthly surveys at each sites from January 2006 to December 2007, near the middle of each month. Water samples from each site were collected near the sediment-water interface and stored in an acid-cleaned polyethylene bottle. Water temperature was measured at the site with a mercury thermometer. In the laboratory, water samples from each site were immediately filtered (0.2 μm membrane filters) and stored at 4°C until analyses. Three sediment cores were collected each month from April 2006 to March 2007. The surface layer (5 cm depth) sediment was sliced and combined into a composite sample at each site. The sediment samples homogenized and stored in a sterilized plastic bag. Samples were then transported in refrigerated coolers to the laboratory for the measurement of potential denitrification within 4 hr. In

Fig. 1 Location of the sampling sites in Meiliang Bay, Taihu Lake, China.

the laboratory, sediment sub-samples obtained in October 2006 were used for sediment physico-chemical characteristics analyses. Wet sediments were freeze-dried and ground, then sieved with a standard 100-mesh sieve, and then dry sediment samples were collected for analyses.

1.3 Sample analyses

Analysis of ammonium and nitrate in the water column took place photometrically with a flow-injection autoanalyzer (Skalar Sanplus, The Netherlands).

Loss on ignition (LOI, %) was measured by calculating the weight loss after heating dry sediment samples to 550°C for 6 hr. Dried aliquots of sediment were homogenized and analyzed for total organic carbon (TOC) and total nitrogen (TN) in a CHN elemental analyzer (CE-440, USA). For total phosphorus (TP) determination, 0.2 g finely ground dry sediments were combusted at 450°C in a muffle furnace for 3 hr, followed by 3.5 mol/L HCl extraction (Ruban et al., 1999). Soluble reactive phosphorus (SRP) concentrations in the extract were analyzed using the Molybdenum Blue method (Murphy and Riley, 1962). The grain sizes were measured using a Mastersizer 2000 Laser Size Analyzer (Malvern Co., UK). The percentages of grain size groups including clay (0.02–4 μm), silt (4–63 μm) and sand fractions (63–500 μm) were determined (Das, 1990).

1.4 Measurements of potential denitrification

Potential denitrification was measured using the acetylene inhibition technique for slurries according to Sørensen (1978) and Magalhães et al. (2005) with some modifications. In brief, the slurries were prepared by adding 10 mL of incubation water to 50 mL serum bottles containing weighed, homogenized wet sediments (appr. 3 g). Serum bottles were hermetically sealed with butyl stoppers and aluminum crimp seals. Each serum bottle, with the sediment and incubation water, was purged with helium to remove O_2. Triplicate samples with and without acetylene (20%, *V/V*) were run and a separate set of time zero samples were sacrificed immediately after acetylene addition. All samples were incubated and simultaneously shaken (70 r/min) in the dark for 4 hr at ambient lake water temperature. At time zero and 4 hr, 18 mL of gas were collected (after headspace equilibration via vigorous shaking) from each serum bottle. The gas samples were collected from each serum bottle by simultaneously adding salt solution and recovering the gas displaced by salt solution addition (Joye et al., 1996). In detail, 18 mL of a 3 mol/L NaCl solution was injected into the serum bottle using a syringe. Simultaneously, 18 mL of gas sample was displaced and collected using another syringe. The gas sample was injected into an evacuated serum vial (18 mL) and stored for later analysis of N_2O. N_2 produced via denitrification was calculated as the difference between the N_2O produced with and without acetylene. N_2O was quantified using a gas chromatograph (Shimadzu GC-14B, Japan) equipped with an electron-capture detector and its concentration was calculated using certified gas standards.
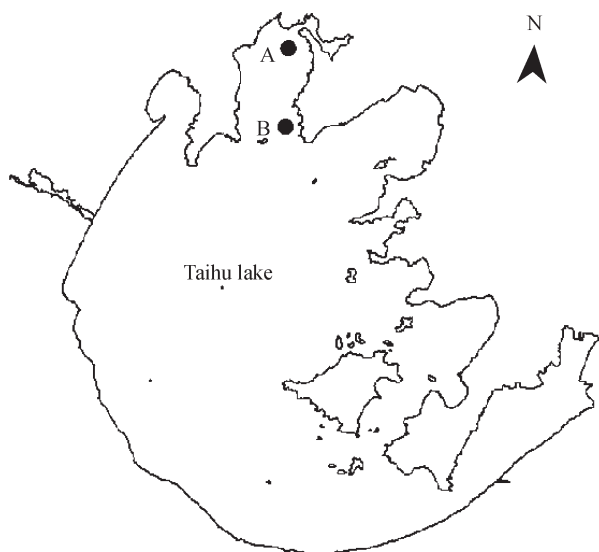
1.5 Influence of environmental factors on denitrification rates

1.5.1 Temperature

The effect of temperature on sediment denitrification rate was investigated on sediments at both sites in October 2006. Sediment samples were collected and slurries were prepared as described above. The samples were incubated under three different temperature regimes (10, 20, 30°C) with lake water enriched with 10 mg/L NO_3^--N and 30 mg/L glucose-C.

1.5.2 Organic carbon and nitrate

The effects of carbon, nitrate and carbon plus nitrate on sediment denitrification rates were investigated for both sites sediments in October 2006. Sediment samples were collected and slurries were prepared as described above. The incubation water treatments were as follows: (1) lake water only (LW treatment); (2) lake water enriched with 30 mg/L glucose-C (LW + C treatment); (3) lake water enriched with 10 mg/L NO_3^--N (LW + N treatment); (4) lake water enriched with 30 mg/L glucose-C and 10 mg/L NO_3^--N (LW + C + N treatment). Incubation temperature was maintained at 20°C for all treatments.

1.6 Statistical analysis

One-way ANOVA was performed to determine significant differences in monthly denitrification rates and between different environmental factor treatments. Linear regression analysis was further used to determine the relationship between denitrification rates and environmental factors. For all statistical methods used herein, results were considered significant if probabilities were less than 0.05.

Statistical analysis was performed using the statistical program SPSS 12.0.

2 Results

2.1 Water column and sediments characteristics

Inorganic nitrogen concentrations in the water column from January 2006 to December 2007 are shown in Fig. 2. NH_4^+-N concentrations varied greatly over time at both sampling sites with obvious seasonal trends (Fig. 2), Mean value of NH_4^+-N concentration in water column in inner bay (site A) was 2.45 mg/L; and generally lower in outer bay (mean value 0.86 mg/L). However, the mean values of NO_3^--N concentrations in the water column were similar between two sites (0.75 mg/L for A and 0.87 mg/L for B); but, there were strong seasonal variations at both sites. Water temperature was similar at both sites, with lowest average temperatures 8°C in winter 2007, 19°C on average in spring and fall, and the highest average temperature in summer 29°C (Fig. 3).

Physico-chemical characteristics of sediments collected in October 2006 are shown in Table 1. The content of organic matter content (LOI % dry weight), TOC and TN were not significantly different between sites, but TP concentration was significantly higher in inner bay (992 mg/kg) than in outer bay (521 mg/kg). Grain size distribution was similar at both sites with the silt fraction (4–63 μm) dominating the distribution (more than 70% particles) and the clay fraction (0.02–4 μm) accounting for less than 30% of the particles. The proportion of sand particles (63–500 μm) was very low, accounting for only 0.6% and 0.5%, in inner bay and outer bay, respectively.
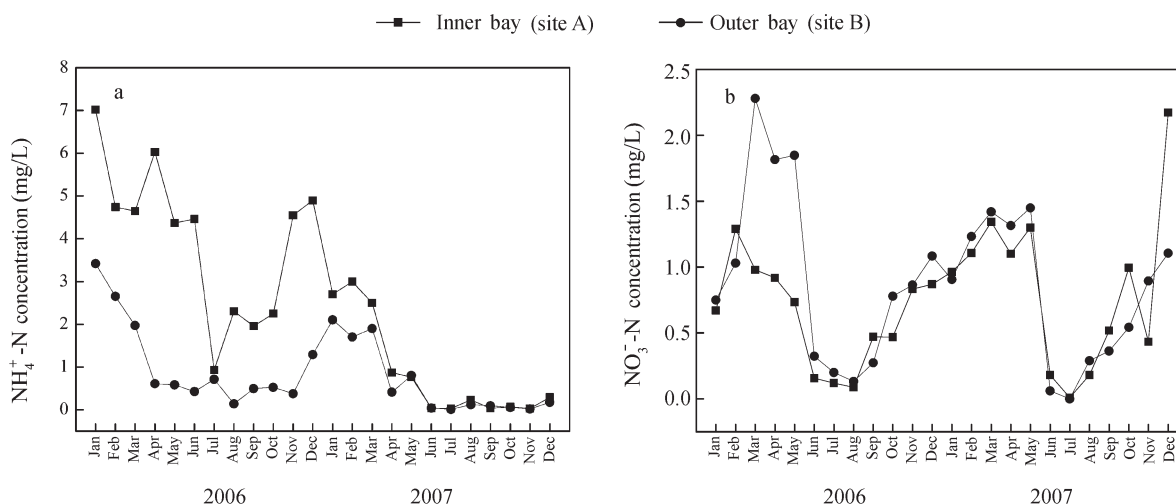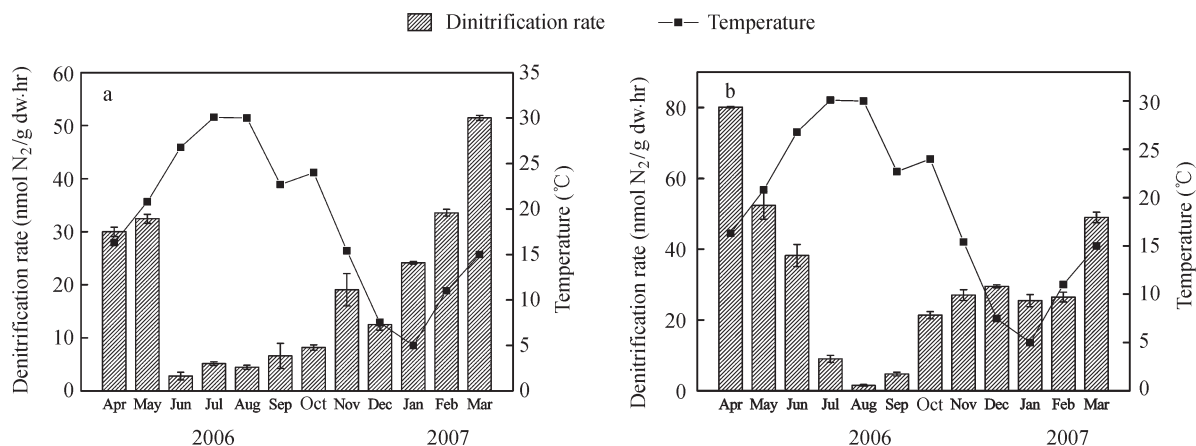

Fig. 2   Monthly NH_4^+-N and NO_3^--N water concentrations from January 2006 to December 2007.

Table 1   Selected physico-chemical characteristics of sediments

Site	LOI (%, *m/m*)	TOC (%, *m/m*)	TN (%, *m/m*)	TP (mg/kg)	Clay (%, *V/V*)	Silt (%, *V/V*)	Sand (%, *V/V*)
Inner bay	3.8	1.5	0.20	992	23.2	76.2	0.6
Outer bay	4.1	1.4	0.21	521	24.4	75.1	0.5

LOI: loss on ignition.

Fig. 3 Mean sediment denitrification rates (± SD) and water temperature of both sites from April 2006 to March 2007. (a) inner bay (site A); (b) outer bay (site B).

2.2 Sediment denitrification

Sediment denitrification rates at both sites varied seasonally (Fig. 3). Denitrification rates ranged from 2.8 to 51.5 nmol N_2/(g dw·hr) in inner bay (Fig. 3a), with the highest rates in March (ANOVA, $p < 0.05$) and significantly low rates in June and August (ANOVA, $p < 0.05$). In outer bay, denitrification rates were similar ranging from 1.5 to 81.1 nmol N_2/(g dw·hr) (Fig. 3b), with the highest rates in April (ANOVA, $p < 0.05$), and significantly low rates in July, August and September (ANOVA, $p < 0.05$). In addition, sediment denitrification rates were positively correlated to NO_3^--N concentrations (for inner bay $R = 0.89$, $p < 0.001$, $n = 12$; Fig. 4a; and for outer bay $R = 0.84$, $p < 0.001$, $n = 12$; Fig. 4b).

2.3 Influence of environmental factors on denitrification rates

2.3.1 Temperature

The relationship between potential denitrification rate and incubation temperature is shown in Fig. 5. For both sites, potential denitrification rates estimated from sediment slurries with excess NO_3^--N increased with temperature (Fig. 5), and were significantly different at studied incubation temperatures ($p < 0.05$).

2.3.2 Organic carbon and nitrate

Denitrification rates at both sites were low when sediments were incubated only with lake water (Fig. 6). No effect of glucose on the denitrification rates was observed for either site in the absence of nitrate additions, but single nitrate addition significantly increased denitrification rates at both sites ($p < 0.05$). In addition, at both sites sediments incubated in the presence of nitrate plus glucose showed denitrification rates which were not significantly different from those with single nitrate treatments ($p > 0.05$).

3 Discussion

3.1 Inorganic nitrogen variation in water column

NH_4^+-N concentrations in inner bay (site A) were generally higher than those in outer bay (site B), which may be attributed to the differences in land use, external and internal loadings. Site A was located at the Liangxihe River discharge point into Meiliang Bay, land use around site A includes tourism, urban land and vegetation coverage. More anthropogenic inputs can be expected at site A. Site B was located at the inlet of Meiliang Bay, land use around outer bay includes agricultural and vegetation coverage. Site B mainly suffers from non-point agricultural pollution.
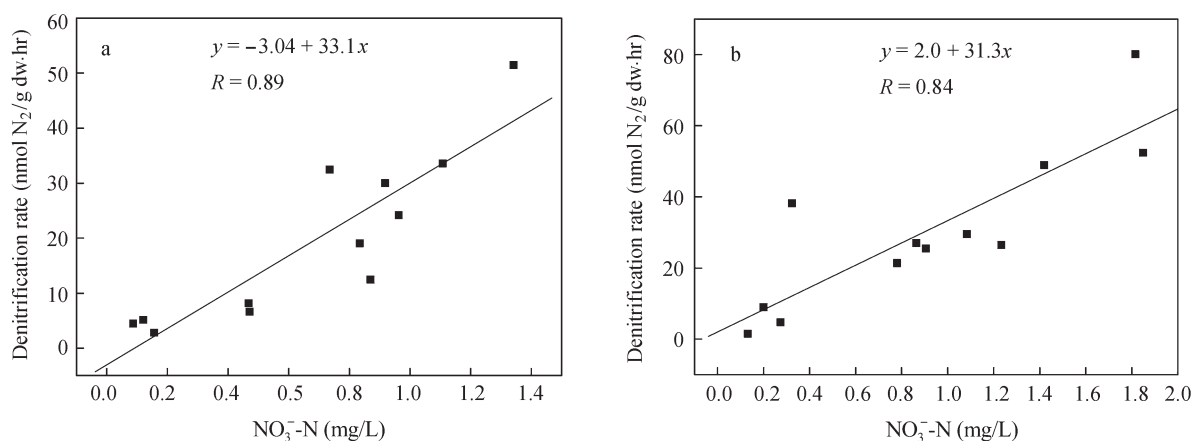
Fig. 4 Linear relationships established for monthly surveys between denitrification rates and NO_3^--N concentration. (a) inner bay; (b) outer bay.
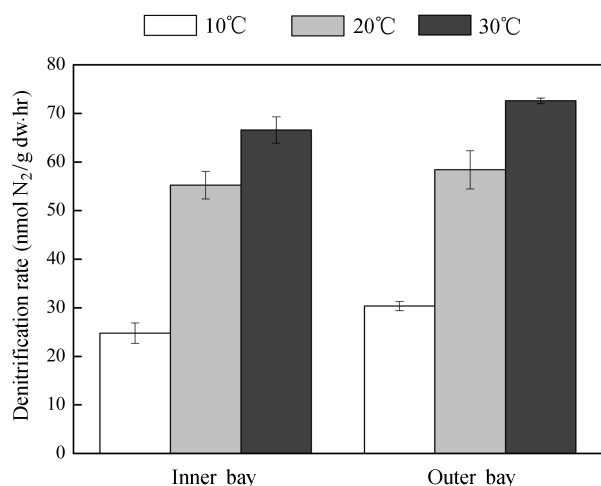
Fig. 5 Effect of temperature on denitrification rates (mean ± SD) in sediments collected in October 2006.
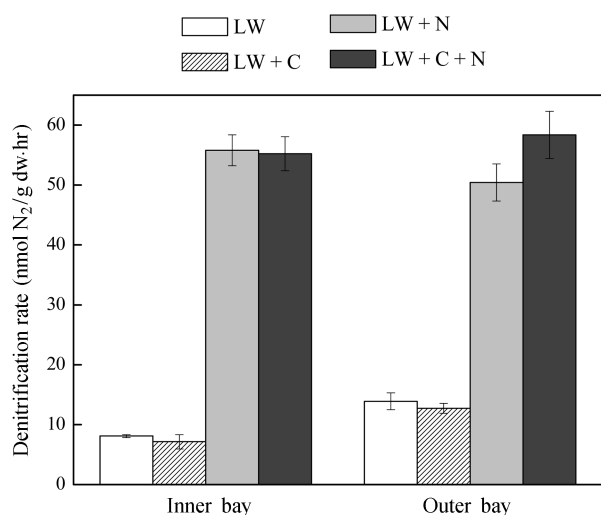
Fig. 6 Effects of glucose and nitrate on denitrification rates (mean ± SD) in the sediments collected in October 2006.

Generally, NO_3^--N concentrations showed little spatial variation, which implied that both sites were similarly affected by anthropogenic nitrogen loading.

There was temporal variation in dissolved inorganic nitrogen in the water column at both sites. Maximum NH_4^+-N concentration occurred in winter (dry season) when biological activity was low and inverse water flow occurred, these results are in agreement with other studies (e.g., Cai et al., 1997; Wang et al., 2007). The possible reason for the high winter NH_4^+-N concentration is that surface and subsurface catchment inputs are high in the non-growing season and microbial activity is simultaneously low due to temperature inhibition (Cai et al., 1997; Wang et al., 2007). NO_3^--N concentrations reached highest values in spring, with a "lag" of about two months, and minimum value occurred in summer (June–August). In general, inorganic nitrogen concentrations were low in the growing season (April–October) and were most likely influenced by biotic factors such as algal uptake, and zooplankton and zoobenthos excretion. This is supported by the observation that phytoplankton biomass is high

from April to October in Meiliang Bay with annual algal blooms which strongly influence nutrient concentration (Zhang et al., 2007). NH_4^+-N is taken up by cyanobacteria more rapidly than NO_3^--N or NO_2^--N, therefore, in spring, NH_4^+-N concentrations decreased markedly while NO_3^--N concentrations remained at high level.

3.2 Denitrification

In monthly assays, denitrification rates at both sites showed clear seasonal trends, with higher rates in spring (March–May) and lower rates in summer and autumn (Fig. 3). Denitrification rates at both sites were positively correlated to NO_3^--N concentration (Fig. 4), suggesting that denitrification rates at both sites were regulated by NO_3^--N availability. This agrees well with previous studies that found maximum denitrification rates when water column NO_3^--N concentrations were the greatest (Ogilive et al., 1997; Silvennoinen et al., 2008a).

The seasonal trend towards a spring (March–May) peak in the denitrification rates have been observed in other sediments (Pattinson et al., 1998; García-Ruiz et al., 1998a), our study showing the importance of nitrate concentration and temperature on denitrification suggests that this peak may be due to the varying importance of nitrate and temperature at different periods of the year. Despite high nitrate concentrations from December to February, denitrification may be expected to be limited by low temperatures. During the spring, denitrification was increasingly favoured by rapidly rising temperatures while nitrate concentrations remained relatively high, however, towards the end of this period nitrate concentration started to decrease, perhaps due to increased phytoplankton uptake, denitrification process and decreased run off (Cai et al., 1997; Zhang et al., 2007). Nitrate concentration remained low until early autumn. In summer and early autumn, denitrification was therefore probably nitrate-limited for much of the time. Despite the high temperatures in this period, the denitrification rates remained low. Nitrate availability in overlying water made the temperature effect on denitrification rates ambiguous (Hasegawa and Okino, 2004).

Denitrification may be influenced by many factors, such as nitrate, reducible organic substances, oxygen concentration and temperature (Pinay et al., 2003; Baeseman et al., 2006; Opdyke and David, 2007). Sediment characteristics such as particle size, water content and organic content can also influence denitrification (García-Ruiz et al., 1998b; Inwood et al., 2007). In this study, denitrification rates showed little spatial variation. This could be due to the combined influence of environmental variables throughout the year. The TP content was the only abiotic characteristic of sediments to vary significantly between sites (Table 1). Therefore, the effect of physico-chemical characteristics of sediment on denitrification rates of the two sites is negligible. The water temperature difference between inner bay and outer bay never exceeded 1°C and therefore was unlikely to be an important factor leading to the denitrification rates difference. The bacterial population size and their metabolism of the two sites are unknown, and we

are unable to identify the effect of bacterial characteristics of sediment on denitrification rates. At this stage, nitrate was considered probably the key factor in determining the denitrification rates of the two sites.

3.3 Influence of environmental factors on denitrification

The denitrification rates at both sites increased with the temperature in the condition test (Fig. 5), suggesting that denitrification in the sediments of Meiliang Bay was limited by temperature. Previous studies using sediment slurries reported the exponential increase of denitrification rates with the investigated temperature ranges of 14–35.5°C for lake sediments (Messer and Brezonik, 1983), 5–18°C for marine sediments (Seitzinger et al., 1984) and 5–25°C for river sediments (García-Ruiz et al., 1998a; Silvennoinen et al., 2008b). Higher water temperature results in elevated microbial metabolism, causing denitrifying bacteria to metabolize NO_3^--N faster (Richardson et al., 2004).

The results of glucose and nitrate amendments indicate that nitrate concentration is likely to be the key factor limiting denitrification rates for both sites. Unamended or glucose amended sediments did not stimulate denitrification in the absence of nitrate (Fig. 6). The role of nitrate in limiting denitrification in the sediments of Meiliang Bay is clearly evident after amendment of the sediments with nitrate. McCarthy et al. (2007) also found that NO_3^--N addition can stimulate the potential denitification in the sediments of Meiliang Bay. Some previous studies have shown that nitrate is usually the most limiting factor in determining denitrification in aquatic ecosystems (García-Ruiz et al., 1998b; Esteves et al., 2001; Magalhães et al., 2005).

Low sedimentary organic carbon contents in sediments may limit potential denitrification rates has been reported previously (Morris et al., 1988; Bradley et al., 1995). Additionally, the type of organic carbon available as an electron donor can influence the potential denitrification in the sediments (Pfenning and McMahon, 1996; Baeseman et al., 2006). In this study, the addition of a high level (30 mg/L C) of the carbon source alone did not lead to a significant increase in denitrification rates at both sites (Fig. 6). The situation was similar when the nitrate plus glucose treatments were examined. For the inner bay sediments, denitrification rates were not significantly different between the treatments with the addition of nitrate and nitrate plus glucose. For the outer bay sediments, the denitrification rate with nitrate plus glucose had a minor increase compared with nitrate alone, but no significant difference was detected. The results implied that organic carbon did not affect the denitrification rates in the sediments of Meiliang Bay, which is consistent with the relatively high content of organic carbon in the sediments (Table 1).

4 Conclusions

In summary, dissolved inorganic nitrogen in the water column showed clear temporal variation. Nitrate is the primary limiting factor in denitrification in the sediments of Meiliang Bay, Taihu Lake. Denitrification occurred with greater rates in the sediments during the spring most likely because available nitrate concentrations were higher then. In the summer and early autumn, denitrification was nitrate-limited for much of the time. Denitrification in the sediments was limited by water temperature, but not regulated by suitable C substrate in the sediment. Nitrate in the water column was depleted during summer and early autumn, and this suppressed the effective removal of nitrogen from Taihu Lake by denitrification.

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